Effects of Ground Loop Currents on Signal Intensities in Electrospray Mass Spectrometry

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The occurrence of electrochemical processes during the operation of an electrospray ionization (ESI) source is well established. In the positive ion mode, electrons are drawn from the ESI metal capillary to a high voltage power supply. These electrons are the product of charge-balancing oxidation reactions taking place at the liquid/metal interface of the ion source. In a recent study, (*Anal. Chem.* **2001,** *73*, 4836–4844), our group has shown that the introduction of a ground loop can dramatically enhance the rate of these oxidation processes. Such a ground loop can be introduced by connecting the sample infusion syringe (or the liquid chromatography column, in the case of LC-MS studies) to ground. The magnitude of the ground loop current can be controlled by the electrolyte concentration in the analyte solution, and by the dimensions of the capillary connecting the syringe needle and the ESI source. Using ferrocene as a model system, it is demonstrated that the introduction of such a ground loop can significantly enhance the signal intensity of analytes that form electrochemically ionized species during ESI. However, analytes that form protonated molecular ions, such as reserpine, also show higher signal intensities when a ground loop is introduced into the system. This latter observation is attributed to the occurrence of electrolytic solvent (acetonitrile and/or water) oxidation processes. These reactions generate protons within the ion source, and thus facilitate the formation of $[M + nH]^{n+}$ ions. Overall, this work provides an example of how the careful control of electrochemical parameters can be exploited to optimize signal intensities in ESI-MS. (J Am Soc Mass Spectrom 2004, 15, 1748–1754) © 2004 American Society for Mass Spectrometry

Electrospray ionization mass spectrometry (ESI-MS) has become an indispensable analytical tool for a wide range of applications [1]. Examples include the analysis of metabolites in the context of drug discovery [2], studies on protein function and dynamics [3, 4], and experiments on large macromolecular assemblies [5]. The hallmark of ESI is the production of intact gas phase ions at atmospheric pressure, directly from analytes in solution. A typical ESI source consists of a metal capillary to which a high voltage is applied. Analyte solution is pumped into this capillary. Electrophoretic charge separation leads to the formation of a Taylor cone at the capillary tip, from which highly charged solvent droplets are emitted. Rapid solvent evaporation and subsequent droplet fission ultimately lead to the formation of analyte ions in the gas phase [6, 7]. These ions are transferred into the vacuum chamber of the mass spectrometer by means of a differentially pumped interface.

This study focuses exclusively on the commonly used positive ion mode, where a positive voltage is applied to the ESI source, such that the droplets emitted from the capillary tip are positively charged. Because the analyte solution delivered to the capillary is electrically neutral, and because electrons are the only charge carriers that can be transported through metal, the ESI process has to be accompanied by charge balancing oxidation processes at the metal-liquid interface of the ion source. The electrons produced in these processes flow from the ESI capillary (anode) to the high voltage power supply, and ultimately to the mass spectrometer (cathode), where they reduce the positive charges emitted from the ESI source, thus completing a series circuit [8]. Usually, the mass spectrometer is connected to ground (i.e., a potential of 0 V), together with the low voltage side of the power supply (Figure 1).

Electrochemical processes occurring in ESI-MS are an area of great interest [9–12]. The ESI source has been characterized as an electrolytic cell, for which the magnitude of the electric current is controlled by the rate of charged droplet production [13–15]. Possible charge balancing reactions include the oxidation of the metal capillary, the solvent, and the analyte(s). In addition, the oxidation of solution impurities can play a role [16]. In the simplest case, the most easily oxidizable species will provide all of the electrons needed to compensate the positive charges emitted from the sprayer tip. Two

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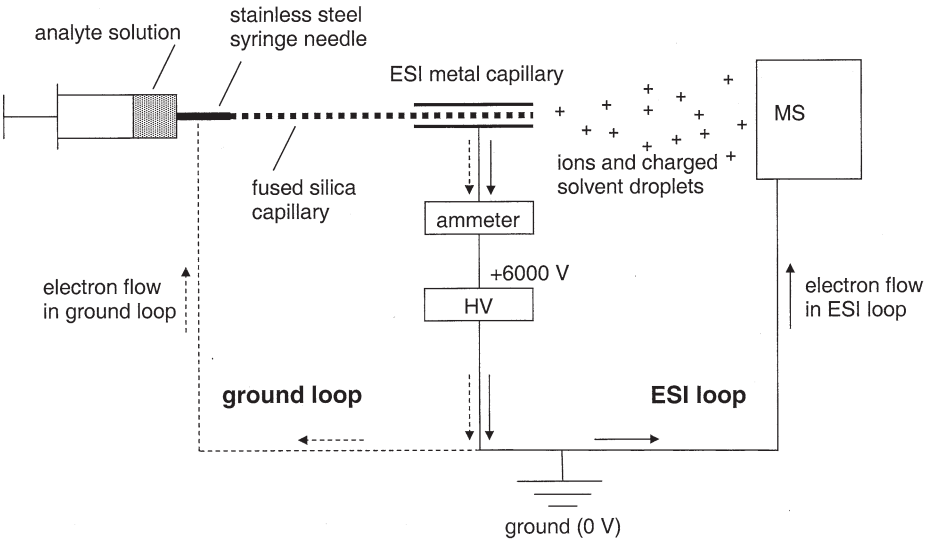

Figure 1. Schematic layout of an electrospray ionization setup, assuming positive ion mode. Electrons in the ESI loop (solid arrows) flow from the ion source to the mass spectrometer (MS). Grounding of the sample infusion syringe introduces a ground loop, corresponding to electron flow that is indicated by the dashed arrows. Electrons flowing in both, the ESI loop and the ground loop have to be supplied by charge-balancing oxidation reactions in the ion source.

or more charge-balancing processes may occur in parallel when the electrochemical conversion of the most easily oxidizable species is limited by material transport [17]. All of these charge-balancing processes are interesting from an analytical point of view because they affect the chemical composition of the solvent droplets emitted from the Taylor cone.

Depending on the characteristics of the analyte in solution, at least three different types of ion formation can be distinguished. (1) Electrochemical ionization can occur when analyte oxidation is one of the charge balancing processes taking place within the ion source. This process often leads to the formation of radical cations from neutral compounds in solution ($M \rightarrow M^{\bullet +} + e^-$), followed by liberation of the $M^{\bullet +}$ ions into the gas phase. This mechanism applies to metallocenes, porphyrins, polyaromatic hydrocarbons, and many other easily oxidizable compounds [18–20]. The production of $M^{\bullet +}$ ions may occur directly at the metal/liquid interface of the ion source. Alternatively, these species may be formed away from the electrode surface, if strong oxidizing agents (e.g., electrochemically oxidized solvent ions) are present in solution. (2) Another way of forming ions during ESI is by protonation. The generation of $[M + nH]^{n+}$ ions is very common for peptides, proteins, and other analytes that possess basic sites. (3) A third possibility is the liberation of preformed ions, which takes place during the ESI-MS analysis of metal cations, quaternary ammonium compounds, and other charged solution phase species [21]. The importance of charge-balancing redox processes taking place in the ESI capillary is most obvious in the case of electrochemically ionized compounds. However, electrochemistry can also affect the signals observed for protonated analytes. For example, the oxidation of water ($2\ H_2O \rightarrow 4\ H^+ + 4\ e^- + O_2$) can be a dominant charge balancing reaction when studying aqueous solutions. Van Berkel et al. have demonstrated that this process can lead to a significant acidification within the ion source. At low flow rates, and by using an ESI capillary made of platinum, a pH drop by up to four units was observed for solutions that were initially neutral [16]. The protons generated by water oxidation can significantly affect the charge states of the observed ions, e.g., for proteins that undergo pH-induced conformational changes [22]. Relatively little is known about the consequences of electrochemical processes for the signals observed for preformed ions, but it might be suspected that any effects in this case are less pronounced than for the previous two types of ionization.

The infusion of analyte solution into the ESI capillary is normally performed by a syringe pump, using a fused silica capillary or PEEK tubing to connect the sample injection device and the ion source. When analyzing solutions that contain electrolytes, such a setup provides an electric connection between the ESI emitter and the stainless steel syringe needle. In order to avoid the exposure to dangerous high voltages, many operators connect the syringe needle to ground. Similar considerations apply when the mass spectrometer is used to analyze the effluent from a liquid chromatography column. In a recent study [22], our laboratory has shown that grounding of the syringe needle introduces a ground loop into the ESI circuit (Figure 1). The resulting system represents two coupled electrolytic cells that share the ESI source as common anode. The mass spectrometer remains the cathode of the ESI loop, while the syringe needle represents the cathode of the

ground loop. Under these conditions, the ion source has to supply electrons to *both* loops of the circuit, thereby increasing the rate of oxidation reactions taking place inside the ESI capillary. The total current measured by the ammeter in Figure 1 is the sum of the ground loop current and the current flowing in the ESI loop.

In this work, we explore the effects of ground loop currents on the signal intensities of three model analytes, representing the different types of ionization that have been discussed above. Ferrocene (bis(cyclopentadienyl) iron, MW 186 Da) is used as an electrochemically ionizable substance. ESI of this compound results in the formation of ferrocene$^{\bullet+}$ radical cations [18]. Reserpine (MW 608 Da) is an indole alkaloid. In ESI-MS, this compound is easily observable as a singly protonated $[M + H]^+$ ion at *m/z* 609. Reserpine is often used as a test analyte to characterize the performance of mass spectrometers [23]. Choline (MW 104 Da) is a quaternary ammonium compound, and it represents a preformed solution phase ion that gets liberated into the gas phase during ESI [24]. It will be shown that the establishment of a ground loop in the ESI circuit can have significant effects on the observed ion intensities, thereby providing an example how electrochemical phenomena can be exploited to optimize signal intensities in ESI-MS.

Experimental

Chemicals

Ferrocene, reserpine and choline chloride were obtained from Sigma (St Louis, MO). Teraethylammonium nitrate was obtained from Fluka (Steinheim, Switzerland). This salt was used as electrolyte because of its solubility in pure acetonitrile as well as in acetonitrile/water mixtures. HPLC grade acetonitrile was supplied by Caledon (Georgetown, ON, Canada). All chemicals were used without further purification unless noted otherwise.

Electrospray Mass Spectrometry

Ions were generated by pneumatically assisted ESI (ion spray) in the positive ion mode at a voltage of +6000 V. Control measurements with a high voltage probe confirmed that the sprayer voltage remained unchanged, even when the current in the ESI circuit was dramatically increased by creating a ground loop in the system. Purified dry air was used as nebulizer gas. Unless noted otherwise, the ESI sprayer capillary used was made of platinum (Small Parts Inc., Miami Lakes, FL; capillary length 6 cm, i.d. 0.21 mm, o.d. 0.41 mm). The analyte solution was delivered to the mass spectrometer by a 1 mL glass syringe equipped with a type 304 stainless steel syringe needle (SGE, Austin TX). The syringe was mounted on a syringe pump (Harvard Apparatus, model 22, Saint Laurent, PQ, Canada), and analyte solution was pumped into the ion source through a fused silica capillary (Polymicro Technologies, Phoenix, AZ; i.d. 75 μm, o.d. 150 μm, with lengths of 13 or 130 cm) at a flow rate of 10 μL/min. One end of the fused-silica capillary was connected to the syringe needle by a PEEK connector (Upchurch Scientific, Oak Harbor, WA), the other end was pushed through the inside of the ESI capillary. For all experiments, the fused-silica capillary was positioned flush with the ESI capillary as indicated in Figure 1. This arrangement resulted in the most stable ESI-MS signals. The distance between the tip of the ESI capillary and the curtain plate of the mass spectrometer (counter electrode) was fixed at 4 cm. The electric current in the ESI circuit current was measured with a Fluka model 8060A multimeter. This battery-powered instrument was floated at the sprayer voltage, well isolated from ground. Analyte signal intensities were recorded on a single quadrupole mass spectrometer (a Sciex "Toby" prototype instrument, Concord, ON, Canada) operated in selected ion monitoring mode, using a dwell time of 10 ms. Groups of 50 data points were averaged for an effective dwell time of 0.5 s.

Results and Discussion

The magnitude of the ground loop current in the ESI circuit depends on the electric resistance of the analyte solution filling the capillary that connects the sample injection syringe and the ESI source (Figure 1). One simple way to modify the resistance of this connection is by changing the electrolyte concentration of the solution. This is demonstrated in Figure 2, where the current drawn from the ESI source is measured for different electrolyte (tetraethylammonium nitrate) concentrations, using acetonitrile as solvent and a 13 cm long fused silica capillary connection. When spraying

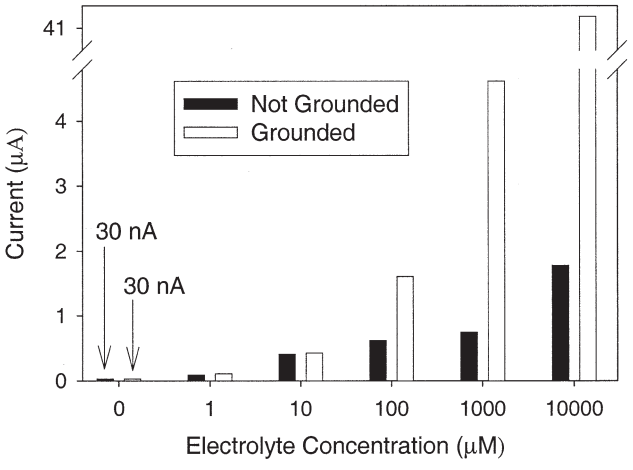

Figure 2. Current drawn from the ESI source as a function of electrolyte ($[N\{C_2H_5\}_4]^+$ NO_3^-) concentration, using acetonitrile as solvent and a 13 cm long fused silica capillary. The two data sets refer to conditions where the sample infusion syringe was connected to ground, or disconnected from ground, respectively.

electrolyte concentrations between 0 and 10 μM, the measured current remains independent of the grounding status of the syringe needle. This indicates that the ground loop current is negligible for such low electrolyte concentrations. At higher concentrations, the current for the non-grounded system increases moderately, while a dramatic increase is seen in the presence of the ground loop. Grounding the syringe needle at an electrolyte concentration of 10 mM was accompanied by a brief arcing event. For safety reasons, and to prevent long-term overload of the high voltage power supply, it was decided to limit the electrolyte concentration for the following experiments to 1 mM. Under these conditions, the current drawn from the ESI source increases sixfold when the syringe needle is grounded, indicating that ~80% of the electrons drawn from the ESI source are flowing through the ground loop at this electrolyte concentration. The data depicted in Figure 2 were obtained for solutions containing 1 mM ferrocene as analyte, however, very similar results were obtained when spraying solutions containing 1 mM reserpine and for analyte-free solutions (data not shown). It is noted that the observed dependence of the measured current for the non-grounded system is consistent with earlier observations [15]. When blocking the ESI source, such that the positively charged spray cannot reach the mass spectrometer interface, the arrangement in Figure 1 allows a direct measurement of the ground loop current. As expected, the currents measured in this way agreed well with the differences between the grounded/not grounded data sets in Figure 2 (data not shown).

ESI mass spectra of ferrocene recorded for the various conditions in this work were very similar to those reported in the literature [18], with ferrocene$^{\bullet+}$ radical cations at *m/z* 186 as the dominant species (data not shown). As in that previous study, the experiments here were carried out by using acetonitrile as solvent. In the absence of added electrolyte, the signal intensity of ferrocene$^{\bullet+}$ is independent of the grounding status of the sample infusion syringe (Figure 3a). Under the conditions of this experiment, 0.2% of the ferrocene being infused into the ion source would have to undergo oxidation to provide the entire electric current of 30 nA. Figure 3b shows that in the presence of 1 mM electrolyte the signal intensity increases about twofold when the syringe needle is grounded. This effect is smaller than the factor of six that might be expected based on the change in the measured current (from 0.75 μA to 4.6 μA). Nonetheless, Figure 3b clearly demonstrates that the introduction of a ground loop can significantly enhance the signal intensity of radical cations produced by oxidation in the ion source. This implies that the increased electron flow drawn from the ion source is balanced by a greater fraction of ferrocene that undergoes oxidation, resulting in a more intense ESI-MS signal for the oxidation product. About 30% of the ferrocene being infused into the ion source would have to undergo oxidation to provide a current of 4.6 μA. However, this estimated percentage only represents an upper limit, because other charge-balancing reactions are likely to play a role as well.

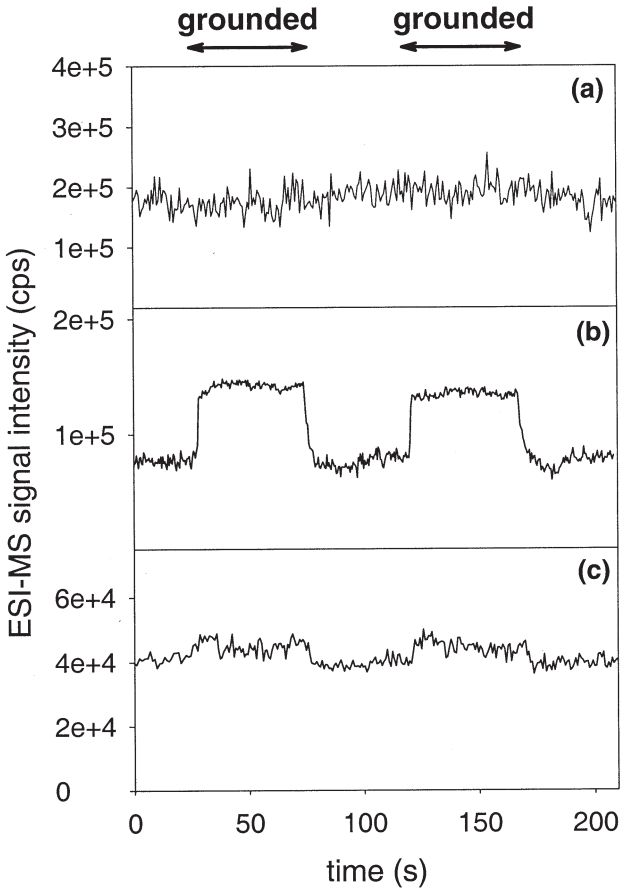

Figure 3. Intensity of ferrocene$^{\bullet+}$ radical cations, generated by electrospraying a 1 mM solution of ferrocene in acetonitrile. The data were measured by selected ion monitoring on a quadrupole mass spectrometer at *m/z* 186. Double-headed arrows indicate the time periods during which the needle of the sample infusion syringe was connected to ground. (**a**) Fused silica capillary length 13 cm, no electrolyte added; (**b**) fused silica capillary length 13 cm, electrolyte concentration 1 mM; (**c**) fused silica capillary length 130 cm, electrolyte concentration 1 mM.

The data depicted in Figure 3a and b were recorded with a relatively short (13 cm) capillary. The magnitude of the ground loop current is expected to depend not only on the electrolyte concentration in the analyte solution, but also on the length of the fused silica capillary connecting the syringe needle and the ion source. Smaller effects are anticipated for longer capillaries, due to the increased electric resistance of the connection. This is confirmed by the data in Figure 3c which were obtained with a capillary length of 130 cm, using an electrolyte concentration of 1 mM. The current increase upon grounding is relatively small, from 0.75 μA to 1 μA. As expected, the concomitant signal increase of the ferrocene$^{\bullet+}$ signal is much less pronounced than for the shorter capillary (Figure 3c).

After having demonstrated the effect of ground loop currents on the signal intensity of ferrocene, we proceeded to carry out analogous experiments on reser-

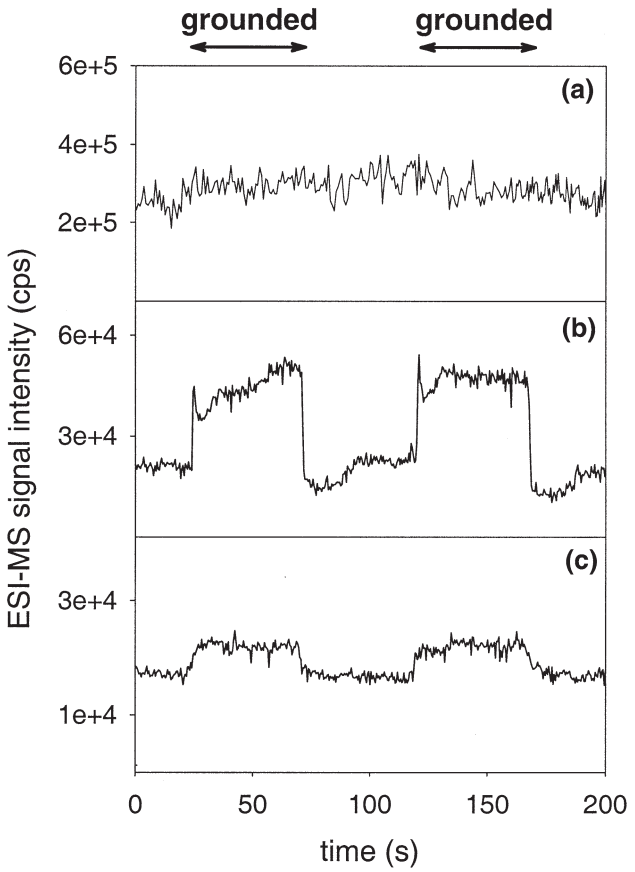

Figure 4. Intensity of $[reserpine + H]^+$ ions (*m/z* 609), generated by electrospraying a 1 mM solution of reserpine in acetonitrile. (**a**) 13 cm fused silica capillary, no electrolyte added; (**b**) 13 cm fused silica capillary, electrolyte concentration 1 mM; (**c**) 130 cm fused silica capillary, electrolyte concentration 1 mM. For more information, see the caption of Figure 3.

pine. As pointed out above, reserpine usually forms $[M + H]^+$ ions during ESI. In a recent study, Van Berkel et al. observed the formation of reserpine oxidation products by incorporating a three-electrode controlled-potential electrochemical cell into the ESI circuit [23]. However, none of these oxidation products were observed under any of the conditions used for this study. Instead, $[reserpine + H]^+$ was the only detectable ionic species. The reserpine data depicted in Figure 4 are analogous to the ferrocene experiments in Figure 3, again using acetonitrile as solvent. Figure 4a shows the signal of protonated reserpine, monitored at *m/z* 609, in the absence of added electrolyte. The grounding state of the syringe does not affect the ion intensity under these conditions. It is interesting to explore the source of protons that is needed for the formation of reserpine ions. We initially suspected water oxidation ($2\ H_2O \rightarrow O_2 + 4\ H^+ + 4\ e^-$) to play a major role in these experiments, as water might be expected to be a ubiquitous contaminant that is easier to oxidize than acetonitrile [12]. However, when the measurement was repeated with carefully dried acetonitrile and reserpine, the observed signal intensities remained virtually unchanged. It therefore appears that a major charge-balancing reaction in these experiments is the electrolytic oxidation of the acetonitrile solvent, to form succinic dinitrile ($2\ CH_3\text{-}CN \rightarrow NC\text{-}CH_2\text{-}CH_2\text{-}CN + 2\ H^+ + 2\ e^-$), as proposed earlier by Cole and co-workers [12]. It is noted that the actual mechanism of acetonitrile oxidation may be more complicated than this simple overall equation implies [25].

In the presence of 1 mM electrolyte, the introduction of a ground loop results in a significant signal enhancement of the $[reserpine + H]^+$ signal. We attribute this effect to a higher proton concentration in the ion source upon establishment of the ground loop, caused by the increased rate of electrolytic solvent oxidation. An increased proton concentration will facilitate the production of protonated analyte ions. Figure 4c shows that, once again, the magnitude of these ground loop effects could be diminished by increasing the fused silica capillary length from 13 to 130 cm.

The results of comparative measurements on ferrocene and reserpine in acetonitrile, carried out in the presence of 5% trifluoroacetic acid, are depicted in Figure 5. In both cases, the fused silica capillary length was 13 cm, and the solutions contained 1 mM electrolyte. The purpose of these measurements was to explore the effects of a major "external" proton source on the ground loop-induced signal changes. Any proton contributions resulting from charge-balancing solvent oxidation in the ion source are expected to be negligible, compared to the protons provided by the acid. For

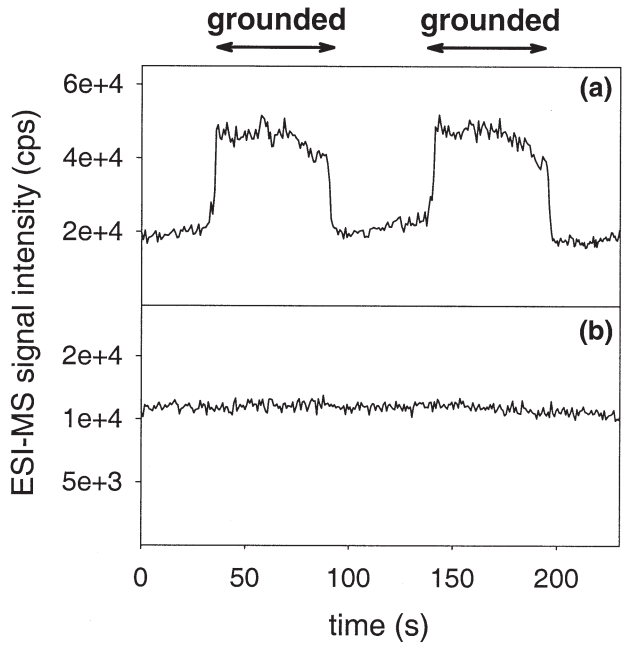

Figure 5. Signal intensities of (**a**) ferrocene$^{\bullet+}$ ions, generated by electrospraying a solution of 1 mM ferrocene in acetonitrile; (**b**) $[reserpine + H]^+$ ions, generated by electrospraying a 1 mM solution of reserpine in acetonitrile. For (**a**) and (**b**) the solutions also contained 5% (vol/vol) trifluoroacetic acid, in addition to 1 mM electrolyte. A 13 cm long fused silica capillary was used in both cases.

ferrocene (Figure 5a), grounding the syringe under these conditions induces a signal enhancement very similar to that observed in the absence of acid (see Figure 3b for comparison). This demonstrates that the ground loop-induced signal enhancement seen for ferrocene is independent of proton concentration changes in the ion source. In the case of reserpine, however, the ground loop effects were completely suppressed by the acid (Figure 5b) This result strongly supports the notion that the protons produced upon solvent oxidation are indeed responsible for the signal intensity changes observed in Figure 4b for reserpine. Interestingly, the signal intensity for reserpine in Figure 5 is lower than that measured in the absence of trifluoroacetic acid (Figure 4b). The reason underlying this observation is not entirely clear, but it could be due to ion suppression effects caused by trifluoroacetate ions.

In an additional set of experiments, the signal intensity of choline was tested. As a quaternary ammonium compound, choline represents a preformed solution-phase ion that forms choline$^+$ (m/z 104) ionic species in the gas phase upon desolvation during ESI. The measured signal intensity was independent of the grounding status of the syringe for all solution conditions tested. This is exemplified in Figure 6 for an electrolyte concentration of 1 mM, and a 13 cm long fused silica capillary. This lack of responsiveness to ground loop currents in the system is not surprising, since neither the proton concentration in solution nor the occurrence of analyte oxidation are expected to play a major role for the formation of gas phase choline ions. The data in Figure 6 represent a control, providing evidence that the signal intensity changes observed for ferrocene (Figures 3b, 5b) and reserpine (Figure 4b) are not due to factors that have been unaccounted for.

As mentioned in the introduction paragraph, one possible charge-balancing reaction in the ESI source is the oxidation of the capillary material [13, 16, 26]. For the experiments described above, this process was eliminated by using an ESI capillary made of platinum.

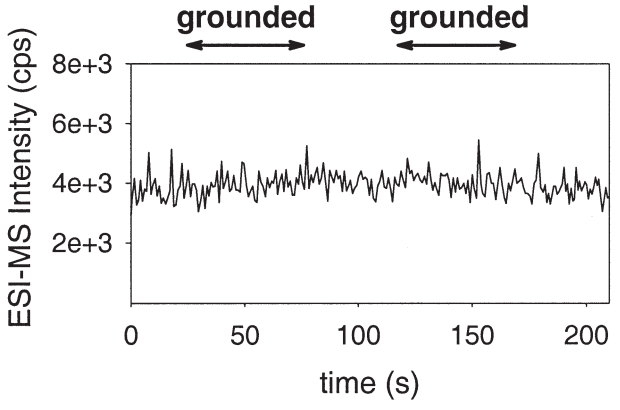

Figure 6. Signal intensity of choline$^+$ ions (m/z 104), generated by electrospraying a solution containing 1 mM choline and 1 mM electrolyte in acetonitrile. A 13 cm long fused silica capillary was used for this experiment.

When the measurements were repeated by using a conventional type 304 stainless steel ESI capillary, the results obtained were virtually indistinguishable from those obtained with platinum. This observation is consistent with the formation of a passivation layer on the steel surface which suppresses further metal oxidation [22, 27]. Another point to consider is that analyte solutions used for ESI-MS often contain a significant percentage of water. For this reason, the experiments were repeated once more, after adding 10% (vol/vol) water to all the acetonitrile solutions. Under these conditions, water oxidation is likely to be a more important charge balancing reaction than the electrolytic decomposition of acetonitrile (note that protons are generated in both processes) [12]. Under these conditions, however, the results obtained were very similar to those described above (data not shown).

Conclusions

It is common practice in ESI-MS to connect the sample injection device (syringe or liquid chromatography equipment) to ground, in order to avoid charging of the equipment and the risk of exposing the operator to high voltages. Presumably, most practitioners of ESI-MS are unaware of the electrochemical consequences of the ground loop that is established in this way. The presence of a ground loop increases the rate of charge balancing oxidation reactions taking place at the metal-liquid interface of the ESI capillary. The present work demonstrates how this effect can be exploited to enhance the intensity of ion signals observed in ESI-MS. For compounds that can be easily oxidized, the increased current drawn from the ESI source causes a larger percentage of the analyte to undergo ionization. For analytes that can be protonated, the increased H^+ concentration in the ion source facilitates the formation of $[M + nH]^{n+}$ ions. The magnitude of the ground loop current depends on the electrolyte concentration in the analyte solution, and on the dimensions of the capillary connection between the ion source and the nearest upstream grounded metal element. In the present work, this metal element was represented by the needle of the sample injection syringe. However, it would also be possible to introduce a separate grounding point further downstream, very close to the ESI emitter. Such an arrangement could provide more flexibility for the design of the ESI setup, as it would remove any restrictions regarding the dimensions of the capillary connecting the sample injection device with the ESI source. An interesting aspect that has not been explicitly addressed in this study is the nature of the electrochemical reduction processes taking place at the liquid/metal interface of the grounding point. The reduced species generated in this way are swept into the ion source where they may undergo partial reoxidation. A detailed investigation of these processes, and their effects on analyte signal intensities in ESI-MS, will be the subject of future studies.

Acknowledgments

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